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| EXAMINER | |
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| DUNSTON, JENNIFER ANN | |
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**Please find below and/or attached an Office communication concerning this application or proceeding.**

The time period for reply, if any, is set in the attached communication.

| Office Action Summary | Application No. | Applicant(s) | |
|---|---|---|---|
| | 10/692,553 | COURT ET AL. | |
| | Examiner | Art Unit | |
| | Jennifer Dunston | 1636 | |

*-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --*

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

1)☒ Responsive to communication(s) filed on *25 May 2007*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims**

4)☒ Claim(s) *1,3-13 and 22-26* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,3-13 and 22-26* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

**Application Papers**

9)☒ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *23 August 2006* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

**Priority under 35 U.S.C. § 119**

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

**Attachment(s)**

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☒ Interview Summary (PTO-413) Paper No(s)/Mail Date. *20070518*.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

## DETAILED ACTION

### *Continued Examination Under 37 CFR 1.114*

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 5/25/2007 has been entered.

Receipt is acknowledged of an amendment, filed 5/25/2007, in which claims 1, 4, 5 and 12 were amended, and claims 24-26 were newly added. Currently, claims 1, 3-13 and 22-26 are pending.

Any rejection of record in the previous office actions not addressed herein is withdrawn.

### *Election/Restrictions*

Applicant elected of Group I without traverse in the reply filed on 12/5/2005. Currently, claims 1, 3-13 and 22-26 are under consideration.

### *Priority*

Applicant's claim for the benefit of a prior-filed application under 35 U.S.C. 119(e) or under 35 U.S.C. 120, 121, or 365(c) is acknowledged. Applicant has not complied with one or more conditions for receiving the benefit of an earlier filing date under 35 U.S.C. 120 as follows:

The later-filed application must be an application for a patent for an invention which is also disclosed in the prior application (the parent or original nonprovisional application or

provisional application). The disclosure of the invention in the parent application and in the later-filed application must be sufficient to comply with the requirements of the first paragraph of 35 U.S.C. 112. See *Transco Products, Inc. v. Performance Contracting, Inc.*, 38 F.3d 551, 32 USPQ2d 1077 (Fed. Cir. 1994).

The disclosures of the prior-filed applications, International Application No. PCT/US01/25507 and Provisional Application Nos. 60/225,164 and 60/271,632, fail to provide adequate support or enablement in the manner provided by the first paragraph of 35 U.S.C. 112 for one or more claims of this application. The prior-filed application numbers do not provide literal or inherent support for the claimed method steps of claims 1-13 and 22. While the prior-filed applications suggest that the disclosed method of homologous recombination may be used to construct complex conditional targeting vectors, the specifications do not set forth the claimed method steps. For example, the prior-filed applications do not provide adequate written description for the method step of using homologous recombination to insert a nucleic acid encoding a selectable marker flanked by a pair of second recombining sites and a first recombining site into a second site into the gene in a bacterial artificial chromosome. The prior-filed applications do not teach how to use the disclosed recombination system to make a vector for the conditional knockout of a gene, where two first recombining sites remain in a gene and recombination of the two first sites produces a nucleic acid sequence that cannot be transcribed to produce a functional protein.

Claims 1-13 and 22-23 have an effective filing date of 2/12/2003.

***Specification***

The disclosure is objected to because of the following informalities: At page 1, paragraph 1 the reference to prior International Application No. PCT/US01/25507 must include the relationship to all nonprovisional applications. It would be remedial to amend the specification to indicate that U.S. Patent Application No. 10/366,044 is a continuation-in-part of International Application No. PCT/US01/25507.

Appropriate correction is required.

***Claim Objections***

Claim 12 is objected to because of the following informalities: the word "recombination" is misspelled in line 3. Appropriate correction is required. This is a new objection, necessitated by the amendment of claim 12 in the reply filed 5/25/2007.

***Response to Amendment – 35 U.S.C. § 1.131***

The declarations filed on 8/23/2006 (signed by Daiguan Yu, Pentao Liu, Donald Court and E-Chiang Lee), 8/28/2006 (signed by Neal Copeland and Nancy Jenkins), and 12/8/2006 (signed by Hilary Ellis) under 37 CFR 1.131 are sufficient to overcome the previous rejections based upon the Casanova et al reference applied under 35 USC § 103(a).

The declarations filed on 8/23/2006 (signed by Daiguan Yu, Pentao Liu, Donald Court and E-Chiang Lee), 8/28/2006 (signed by Neal Copeland and Nancy Jenkins), and 12/8/2006 (signed by Hilary Ellis) under 37 CFR 1.131 have been considered but are ineffective to

overcome the Casanova et al reference applied to new claims 24-26 under 35 USC § 103(a) in the present action (see new rejections presented below).

The evidence submitted is insufficient to establish a reduction to practice of the invention in this country or a NAFTA or WTO member country prior to the effective date of the Casanova et al reference (Genesis, Vol. 32, No. 2, pages 158-160, published online 2/13/2002). The declaration and attached exhibits have been fully reviewed. The declaration attempts to provide evidence of an actual reduction to practice prior to the effective date of the Casanova et al reference. Upon careful review, it has been determined that the declaration does not provide evidence that the claimed method had been reduced to practice prior to 2/13/2002. The declaration does not provide evidence that a nucleic acid encoding a selectable marker flanked by a second pair of recombining sites and a first recombining site was used for homologous recombination. The vectors of the declaration appear to contain only a pair of LoxP sites flanking a selectable marker. These LoxP sites correspond to the selectable marker flanked by a pair of first recombining sites and the selectable marker flanked by a second pair of recombining sites; however they lack the required first recombining site in combination with the second recombining sites, which is required by claim 26. Accordingly, the declaration does not provide evidence that a nucleic acid encoding a selectable marker flanked by a second pair of recombining sites and a first recombining site was made and used in the claimed invention prior to the effective date of the Casanova et al reference. Accordingly, the declaration is insufficient.

***Claim Rejections - 35 USC § 103***

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 24-26 are rejected under 35 U.S.C. 103(a) as being unpatentable over Casanova et al (Genesis, Vol. 32, No. 2, pages 158-160, Published Online 2/13/2002, cited in a prior action; see the entire reference) in view of Lee et al (Genomics, Vol. 73, pages 56-65, 2001, cited in a prior action; see the entire reference). This rejection was made in the Office action mailed 2/26/2007 over claims 1, 3-10, 12, 13, 22 and 23 and has been rewritten to address new claims 24-26, added in the amendment to the claims in the reply filed 5/25/2007.

Casanova et al teach a method for generating a vector for conditional knockout of a gene, comprising the following steps: (i) co-electroporating a BAC construct and a kanamycin cassette flanked by two LoxP sites (LoxP-Kan-LoxP) into *E. coli* JC8679 competent cells, (ii) selecting for kanamycin resistant clones, (iii) transforming the BAC DNA, from a bacterial colony that had undergone homologous recombination, into Cre-expressing bacteria to excise the nucleic acid encoding the selectable marker, which leaves a single LoxP site in the gene (iv) co-electroporating into *E. coli* JC8679 competent cells the BAC DNA comprising the single LoxP site and a plasmid comprising a FRT-PGK$_{Tn5}$neo-FRT-loxP flanked by two homology arms, and (v) transforming the resulting recombinant BAC into FLP-expressing bacteria to excise the marker gene (e.g. page 158, left column, 2$^{nd}$ full paragraph; page 158, paragraph bridging columns; Figure 1). Casanova et al teach the use of ET-cloning (homologous recombination in

*E. coli* to insert the nucleic acid molecules encoding a selectable marker into the BAC construct (e.g. Figure 1). Casanova et al teach that the recombination of the remaining two first recombining sites will produce a nucleic acid sequence that cannot be transcribed to produce a functional protein (e.g. page 158, left column, 2nd paragraph). Casanova et al teach the abovementioned method, where the first recombining sites comprise a LoxP site, and the second recombining sites comprise a FRT site. Casanova et al teach the use of markers that confer resistance of the cell to an antibiotic such as kanamycin (e.g. Figure 1). Further, Casanova et al teach the use of Cre-expressing and Flp-expressing bacteria as taught by Bucholz et al (1996).

Casanova et al do not teach homologous recombination wherein the cell comprises the pL promoter operably linked to a nuclei acid encoding Beta, Exo and Gam and wherein the first recombination site comprise a FRT site.

Lee et al teach a PL operon encoding beta, exo and gam under the control of the temperature-sensitive λ repressor (allele cI857) for use in BAC engineering (e.g. page 56, right column, 1st full paragraph; page 57, left column, 1st full paragraph). Further, Lee et al teach the use of the recombination system in combination with the flpe gene under the control of the $P_{BAD}$ inducible promoter (e.g. strain EL250; Table 1). Lee et al teach that the recombination system is highly efficient and can produce recombination frequencies that are at least 50- to 100-fold higher than those obtained with plasmid based systems (e.g. page 64, left column, last paragraph). Lee et al teach the recombination of an FRT-kan-FRT cassette into the mouse *Eno2* gene within a BAC vector (e.g. page 57, *Construction of plasmids*; Figure 1). Lee et al teach that the use of flpe provides a higher recombination frequency than the original flp gene (e.g. page

60, left column, 1st paragraph). With regard to the use of the recombination system for the construction of conditional targeting vectors, Lee et al state the following:

> This recombination system also facilitates the generation of complicated conditional targeting vectors. While the generation of such vectors often used to take several months, it can now be performed in only a few weeks. The ability to express reversibly Cre or Flpe recombinases in *E. coli* speeds this process even further. A selectable marker flanked with *loxP* or *FRT* sites can now be introduced into an intron of a gene and then be removed by transient Cre of Flpe expression, leaving behind a solo *loxP* or *FRT* site in the intron. See page 64, right column, 2nd paragraph.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the method for generating a vector for conditional knockout of a gene of Casanova et al to include the phage lambda recombination system taught by Lee et al because Casanova et al and Lee et al teach it is within the ordinary skill in the art to use homologous recombination in *E. coli* to engineer BAC vectors to produce conditional targeting constructs. Further, it would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the method to use FRT sites as the first recombination site and LoxP as the second recombination site because Casanova et al teach it is within the ordinary skill in the art to use of a LoxP-Kan-LoxP cassette to insert a single LoxP site and Lee et al teach it is within the ordinary skill of the art to use a FRT-Kan-FRT cassette to insert an FRT site into the intron of a gene. Further, Lee et al teach that the LoxP and FRT sites can be used interchangeably.

One would have been motivated to make such a modification in order to receive the expected benefit of increased efficiency of homologous recombination and FRT site-specific recombination, which would decrease the amount of time required to make the targeting construct, as taught by Lee et al. Further, one would have been motivated to use FRT in place of LoxP and LoxP in place of FRT to have more options in the vector design and subsequent

knockout of the gene by expressing cre or flpe in a targeted mouse, for example. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 24-26 are rejected under 35 U.S.C. 103(a) as being unpatentable over Casanova et al (Genesis, Vol. 32, No. 2, pages 158-160, Published Online 2/13/2002, cited in a prior action; see the entire reference) in view of Stewart et al (US Patent No. 6,355,412, cited in a prior action; see the entire reference). This rejection was made in the Office action mailed 2/26/2007 over claims 1, 3, 4, 6-8, 10-13, 22 and 23 and has been rewritten to address new claims 24-26, added in the amendment to the claims in the reply filed 5/25/2007.

The teachings of Casanova et al are described above and applied as before.

Casanova et al do not teach homologous recombination wherein the cell comprises the pL promoter operably linked to a nuclei acid encoding Beta, Exo and Gam and wherein the first recombination sites comprise a FRT site.

Stewart et al teach a method of performing homologous recombination in a host cell, comprising introducing a nucleic acid sequence encoding RecE/T or Redα/β recombinase (i.e. Lambda Exo and Beta) into a host cell, introducing a polynucleotide comprising a nucleotide sequence homologous to the nucleotide sequence of interest into the host cell, activating the expression of RecE/T, and selecting a cell from the population in which homologous recombination has occurred (e.g. column 28, lines 10-50; column 29, lines 9-35; column 28, line 51 to column 29, line 8; columns 25-27; paragraph bridging columns 37-38). Further, Stewart et

al teach the use of Gam in addition to Exo and Beta or RecE/T (e.g. column 25, lines 5-28; Example 1). Stewart et al teach that a variety of host-vector systems may be utilized to introduce and express the protein-coding sequence of RecE/T or Redα/β, including prokaryotic and eukaryotic cells such as bacterial, yeast, plant, rodent, mice, human, insect or mammalian cells (e.g. column 28, lines 10-40). With respect to regulatory controls, Stewart et al teach that a range of different expression levels and a variety of regulatory sequences are known in the art and the ability to generate a wide range of expression is advantageous for utilizing the method (e.g. column 25, lines 5-44; column 24, line 50 to column 25, line 3). Stewart et al teach that the expression can be regulated by the $P_L$ promoter of phage λ and the inducible lambda repressor $CI_{857}$ (e.g. column 26, lines 1-27). Stewart et al teach that the nucleotide sequence of interest may be extrachromosomal and located on a bacterial artificial chromosome (e.g. column 20, lines 37-57; paragraph bridging columns 28-29). Moreover, Stewart et al teach that the lambda recombinases can be used to achieve high-efficiency targeted cloning (e.g. column 11, lines 3-47).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the method for generating a vector for conditional knockout of a gene of Casanova et al to include the lambda beta, exo and gam genes operably linked to the pL promoter as taught by Stewart et al because Casanova et al and Stewart et al teach it is within the ordinary skill in the art to use homologous recombination to modify BAC constructs in a cell. Further, it would have been obvious to use a FRT site as the first recombining site, because Casanova et al teach the use of both LoxP and FRT sites for the same purpose.

One would have been motivated to make such a modification in order to receive the expected benefit of being able to conduct high efficiency recombination in a variety of host cell types as taught by Stewart et al. Further, one would have been motivated to use FRT in place of LoxP and LoxP in place of FRT to have more options in the vector design and subsequent knockout of the gene by expressing cre or flpe in a targeted mouse, for example. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 1, 3-10, 12, 13, 22 and 23 are rejected under 35 U.S.C. 103(a) as being unpatentable over Rajewsky et al (J. Clin. Invest. Vol. 98, No. 3, pages 600-603, August 1996; see the entire reference) in view of Lee et al (Genomics, Vol. 73, pages 56-65, 2001, cited in a prior action; see the entire reference). This is a new rejection, necessitated by the amendment of the claims in the reply filed 5/25/2007.

Rajewsky et al teach a method for generating a vector for conditional knockout of a gene in a cell, comprising the steps of (i) using homologous recombination to insert a nucleic acid construct encoding a first loxP site and a selectable marker flanked with loxP sites, (ii) excising the selectable marker with Cre, a recombinase specific for the loxP recombining sites flanking the selectable marker, wherein recombination of the remaining recombining sites produces a nucleic acid sequence that cannot be transcribed to produce a functional protein, thereby generating the vector for conditional knockout of the gene in the cell (e.g., paragraph bridging pages 6001-602; page 602, left column, 1st full paragraph; Figure 1A).

Rajewsky et al do not teach two separate steps for the introduction of the three loxP sites in that Rajewsky et al do not teach using homologous recombination to insert a nucleic acid encoding a first selectable marker flanked by a pair of first recombining sites into a first site in a gene in a bacterial artificial chromosome, excising the selectable marker with a first recombinase, and using homologous recombination to insert a nucleic acid encoding a second selectable marker flanked by a pair of second recombining sites. Further, Rajewsky et al do not teach the cell comprising a de-repressible promoter operably linked to a nucleic acid encoding Beta, Exo and Gam, wherein using homologous recombination comprises de-repressing the de-repressible promoter, thereby inducing the expression of Beta, Exo and Gam.

Lee et al teach a PL operon encoding beta, exo and gam under the control of the temperature-sensitive λ repressor (allele cI857) for use in BAC engineering (e.g. page 56, right column, 1st full paragraph; page 57, left column, 1st full paragraph). Further, Lee et al teach the use of the recombination system in combination with the flpe gene under the control of the $P_{BAD}$ inducible promoter (e.g. strain EL250; Table 1). Lee et al teach that the recombination system is highly efficient and can produce recombination frequencies that are at least 50- to 100-fold higher than those obtained with plasmid based systems (e.g. page 64, left column, last paragraph). Lee et al teach the recombination of an FRT-kan-FRT cassette into the mouse *Eno2* gene within a BAC vector (e.g. page 57, *Construction of plasmids*; Figure 1). Lee et al teach that the use of flpe provides a higher recombination frequency than the original flp gene (e.g. page 60, left column, 1st paragraph). With regard to the use of the recombination system for the construction of conditional targeting vectors, Lee et al state the following:

> This recombination system also facilitates the generation of complicated conditional targeting vectors. While the generation of such vectors often used to

> take several months, it can now be performed in only a few weeks. The ability to express reversibly Cre or Flpe recombinases in *E. coli* speeds this process even further. A selectable marker flanked with *loxP* or *FRT* sites can now be introduced into an intron of a gene and then be removed by transient Cre of Flpe expression, leaving behind a solo *loxP* or *FRT* site in the intron. See page 64, right column, 2nd paragraph.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the method for generating a vector for conditional knockout of a gene of Rajewsky et al to include the phage lambda recombination system and bacterial artificial chromosome (BAC) modification taught by Lee et al, because Rajewsky et al and Lee et al teach it is within the ordinary skill in the art to use homologous recombination in to produce conditional targeting constructs and Lee et al teach that BACs can be used as targeting substrates. Further, it would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the single step of homologous recombination of Rajewsky et al to be two separate steps, where the first loxP site is inserted by a separate homologous recombination event followed by site-specific recombination to remove the marker, and where the second pair of loxP sites flanking the selectable marker are introduced by a homologous recombination event, because the application of the method of Lee et al would result in the same structure as taught by Rajewsky et al, and Lee et al specifically teach that the system can be used to make conditional knockout vectors. Moreover, it would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the method to use FRT sites in place of loxP as the second recombination site because Lee et al teach it is within the ordinary skill of the art to use a FRT-Kan-FRT cassette to insert an FRT site into the intron of a gene. Further, Lee et al teach that the LoxP and FRT sites can be used interchangeably.

One would have been motivated to make such a modification in order to receive the expected benefit of increased efficiency of homologous recombination and FRT site-specific recombination, which would decrease the amount of time required to make the targeting construct, as taught by Lee et al. Further, one would have been motivated to use FRT in place of LoxP and LoxP in place of FRT to have more options in the vector design and subsequent knockout of the gene by expressing cre or flpe in a targeted mouse, for example. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 1, 3-13, 22 and 23 are rejected under 35 U.S.C. 103(a) as being unpatentable over Rajewsky et al (J. Clin. Invest. Vol. 98, No. 3, pages 600-603, August 1996; see the entire reference) in view of Muyrers et al (TRENDS in Biochemical Sciences, Vol. 26, No. 5, May 2001, cited on the IDS filed 10/23/2003; see the entire reference) and Stewart et al (US Patent No. 6,355,412, cited in a prior action; see the entire reference). This is a new rejection, necessitated by the amendment of the claims in the reply filed 5/25/2007.

The teachings of Rajewsky et al are described above and applied as before.

Rajewsky et al do not teach two separate steps for the introduction of the three loxP sites in that Rajewsky et al do not teach using homologous recombination to insert a nucleic acid encoding a first selectable marker flanked by a pair of first recombining sites into a first site in a gene in a bacterial artificial chromosome, excising the selectable marker with a first recombinase, and using homologous recombination to insert a nucleic acid encoding a second

selectable marker flanked by a pair of second recombining sites. Further, Rajewsky et al do not teach the cell comprising a de-repressible promoter operably linked to a nucleic acid encoding Beta, Exo and Gam, wherein using homologous recombination comprises de-repressing the de-repressible promoter, thereby inducing the expression of Beta, Exo and Gam.

Muyrers et al suggest the modification of bacterial artificial chromosomes to generate mouse knockout constructs (e.g., page 325, right column). Further, Muyrers et al teach that recombinogenic engineering often involves two rounds of processing, where the first round uses homologous recombination to generate an initial product by integration of a selectable gene, together with additional functional elements at the intended site, and the second round makes use of the extra functional elements to remove the selectable gene, thereby generating the final product (e.g., page 325, right column). Muyrers et al teach that the application of this process can be used to accomplish virtually any DNA alteration (e.g., page 325, right column). Muyrers et al specifically teach the use of site-specific recombination sites that are recombination sites for Cre or FLP recombinases to flank selectable cassettes to leave a single site-specific recombination target site at a particular location (e.g., page 326, paragraph bridging columns; Figure 1(c)). Moreover, Muyrers et al teach that this is a highly efficient way to eliminate the selectable cassette and it is also a useful method of placing a site-specific recombination site exactly where it is required (e.g., page 326, right column). Muyrers et al teach that recombinogenic engineering can be carried out by lambda Beta (Redβ), lambda Exo (Redα) and lambda Gam (Redγ) (e.g., pages 328-329, Recombinogenic engineering using ET recombination). Muyrers et al teach that it is desirable to limit the recombinogenic window by

regulating the expression of one or more of the components of the recombination system (e.g., pages 329-330, Limiting the recombinogenic window).

Stewart et al teach a method of performing homologous recombination in a host cell, comprising introducing a nucleic acid sequence encoding RecE/T or Redα/β recombinase (i.e. Lambda Exo and Beta) into a host cell, introducing a polynucleotide comprising a nucleotide sequence homologous to the nucleotide sequence of interest into the host cell, activating the expression of RecE/T, and selecting a cell from the population in which homologous recombination has occurred (e.g. column 28, lines 10-50; column 29, lines 9-35; column 28, line 51 to column 29, line 8; columns 25-27; paragraph bridging columns 37-38). Further, Stewart et al teach the use of Gam in addition to Exo and Beta or RecE/T (e.g. column 25, lines 5-28; Example 1). Stewart et al teach that a variety of host-vector systems may be utilized to introduce and express the protein-coding sequence of RecE/T or Redα/β, including prokaryotic and eukaryotic cells such as bacterial, yeast, plant, rodent, mice, human, insect or mammalian cells (e.g. column 28, lines 10-40). With respect to regulatory controls, Stewart et al teach that a range of different expression levels and a variety of regulatory sequences are known in the art and the ability to generate a wide range of expression is advantageous for utilizing the method (e.g. column 25, lines 5-44; column 24, line 50 to column 25, line 3). Stewart et al teach that the expression can be regulated by the $P_L$ promoter of phage λ and the inducible lambda repressor $CI_{857}$ (e.g. column 26, lines 1-27). Stewart et al teach that the nucleotide sequence of interest may be extrachromosomal and located on a bacterial artificial chromosome (e.g. column 20, lines 37-57; paragraph bridging columns 28-29). Moreover, Stewart et al teach that when the recombination method is used in combination with site-specific recombination sites, the site-

specific recombinase, which recognizes the sites is under the control of an inducible promoter such that upon induction of recombination expression, recombination between the site-specific recombination sites occurs (e.g., Figure 4). Stewart et al teach that the lambda recombinases can be used to achieve high-efficiency targeted cloning (e.g. column 11, lines 3-47).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the method for generating a vector for conditional knockout of a gene of Rajewsky et al to include the phage lambda recombination system and bacterial artificial chromosome (BAC) modification taught by Muyrers et al, because Rajewsky et al and teach it is within the ordinary skill in the art to use homologous recombination in to produce conditional targeting constructs and Muyrers et al teach that lambda-mediated recombination can be used to modify BACs for mouse targeting constructs or to accomplish virtually any DNA alteration. Further, it would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the single step of homologous recombination of Rajewsky et al to be two separate steps, where the first loxP site is inserted by a separate homologous recombination event followed by site-specific recombination to remove the marker, and where the second pair of loxP sites flanking the selectable marker are introduced by a homologous recombination event, because the application of the method of Muyrers et al would result in the same structure as taught by Rajewsky et al, and Muyrers et al specifically teach that the system can be used to make virtually any alterations. Moreover, it would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the method to use FRT sites (recognized by Flp recombinase) or loxP sites (recognized by Cre recombinase). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the expression of the

lambda-mediated recombination proteins such that they are under the control of the de-repressible $P_L$ promoter of phage λ, because Muyrers et al teach that regulatable expression is desirable, and Stewart et al teach the use of the $P_L$ promoter of phage λ to regulate the expression of the lambda recombination proteins. It would have also been obvious to perform the recombination events in a prokaryotic cell or eukaryotic cell as taught by Stewart et al, because Muyrers et al teach the use of lambda-mediated recombination, and Stewart et al teach that this method can be performed in prokaryotic or eukaryotic cells.

One would have been motivated to make such a modification in order to receive the expected benefit of being able to conduct high efficiency recombination in a variety of host cell types as taught by Muyrers et al and Stewart et al to arrive at a conditional targeting vector with the structure taught by Rajewsky et al. Further, one would have been motivated to use FRT in place of LoxP and LoxP in place of FRT to have more options in the vector design and subsequent knockout of the gene by expressing cre or flpe in a targeted mouse, for example. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

***Response to Arguments - 35 USC § 103***

Applicant's arguments, see page 7, filed 5/25/2007, with respect to the rejection of claims 1, 3-10, 12, 13, 22 and 23 have been fully considered and are persuasive. The previous rejection of claims 1, 3-10, 12, 13, 22 and 23 under 35 U.S.C. 103(a) as being unpatentable over Casanova et al in view of Lee et al, as evidenced by Buchholz et al, has been withdrawn.

With respect to the rejection of claims 24-26 under 35 U.S.C. 103(a) as being unpatentable over Casanova et al in view of Lee et al, Applicant's arguments filed 5/25/2007 have been fully considered but they are not persuasive.

The response essentially asserts that the Casanova et al publication is removed as a reference by the declaration under 37 C.F.R. § 1.131, and the other references of record do not teach or suggest the claimed invention. This is not found persuasive, because the declaration does not provide sufficient evidence to demonstrate an actual reduction to practice prior to the date of the Casanova et al reference for the reasons set forth above in response to the declaration under 37 C.F.R. § 1.131.

Applicant's arguments, see pages 7-8, filed 5/25/2007, with respect to the rejection of claims 1, 3, 4, 6-8, 10-13, 22 and 23 under 35 U.S.C. 103(a) as being unpatentable over Casanova et al in view of Stewart et al, as evidenced by Buchholz et al, have been fully considered and are persuasive. The previous rejection of claims 1, 3, 4, 6-8, 10-13, 22 and 23 has been withdrawn.

With respect to the rejection of claims 24-26 under 35 U.S.C. 103(a) as being unpatentable over Casanova et al in view of Stewart et al, Applicant's arguments filed 5/25/2007 have been fully considered but they are not persuasive.

The response essentially asserts that the Casanova et al publication is removed as a reference by the declaration under 37 C.F.R. § 1.131, and the other references of record do not teach or suggest the claimed invention. This is not found persuasive, because the declaration does not provide sufficient evidence to demonstrate an actual reduction to practice prior to the

date of the Casanova et al reference for the reasons set forth above in response to the declaration under 37 C.F.R. § 1.131.

***Conclusion***

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Jennifer Dunston whose telephone number is 571-272-2916. The examiner can normally be reached on M-F, 9 am to 5 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Joseph Woitach can be reached at 571-272-0739. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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